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Title:

ARRANGEMENT FOR SEPARATING EXCITATION LIGHT AND EMISSION LIGHT IN A MICROSCOPE

[0001] Figs. 1 to 3 illustrate in exemplified manner arrangements according to the invention.

[0002] In Fig. 1 by means of a mirror SP and a beam splitter ST the light (excitation light) of two lasers L1, L2 having different wavelengths is fed into a common beam path, which is reflected on the side S1 of a vapourized prism in the direction of an AOTF (acousto-optical tunable filter). The excitation light is introduced into the AOTF and light diffracted in the first order for the wavelength set by means of the AOTF control frequency is deflected precisely in the direction of a pinhole PH with upstream and downstream pinhole optics PHO for adjusting the beam profile, whereas other possible wavelengths traverse undiffracted in zero order the AOTF and do not reach the pinhole.

[0003] Here the pinhole PH serves simultaneously as an excitation and detection pinhole. By means of scanner units SC1, SC2 and a scanning optics SCO the excitation light is imaged towards a sample in the direction of a microscope beam path M1.

[0004] The light emitted by the sample and comprising fractions of the excitation light and wavelength-shifted fluorescence fractions, passes through the light path in the opposite direction up to the AOTF. By first order diffraction the wavelength fractions of the excitation light once again reach the mirror side S1 of prism PS, whereas the fluorescence fractions traverse the AOTF undiffracted in zero order and consequently assume an angle to the reflected excitation light. Between the returning beams of zero and first order is now precisely located the peak between the prism faces S1 and S2, so that the fluorescence light impinges on side S2 and is reflected by the latter in the direction of a detection unit, here in exemplified manner

comprising a line filter LF, a colour divider NFT and two detectors for different wavelengths.

[0005] As a result of the low AOTF band width of approximately 2 nm for the excitation light it acts as an extreme edge filter with clear advantages compared with dichroic filters with band widths greater than 10 nm.

[0006] This is of particular significance, because the spacing between the excitation wavelength and the fluorescence wavelengths can be smaller than 10 nm and as a result of the arrangement according to the invention a wavelength-dependent separation is still possible.

[0007] By changing the frequency the AOTF can be switched from the wavelength of laser L1 to the wavelength of laser L2 and once again the excitation light can be separated from the fluorescent light.

[0008] In place of the prism with sides S1, S2, it is also possible to use two independent mirrors, which correspond to the sides S1, S2 but which are unconnected. An advantage is that they can also be constructed in rotary manner in order to permit a precise setting to the AOTF or the detection DE.

[0009] Fig. 2 shows a similar arrangement with only a single scanner SC. Here in place of the prism is provided a mirror S, which deflects the excitation light in the direction of the AOTF in the same way as in fig. 1. Here the fluorescent light returning in the zero order through the AOTF passes alongside the mirror S and in this way passes towards a not shown detection.

[00010] Fundamentally arrangements are also conceivable in which the AOTF alone can serve as the separation unit for the excitation light and fluorescent light, in that the laser light passes in the first order direction without an upstream element into the AOTF and the detector light leaves the AOTF at an angle to the excitation light

and passes directly into a detection unit, which only has effects on the length of the construction, because the angle of e.g. 4° is very small and heterodyning of wavelength fractions should be avoided. In addition, a separating mirror may only be provided for the fluorescent light.

[00011] Fig. 3 shows another advantageous construction in the form of an unvapourized prism, which by refraction introduces the light of an excitation laser in the first order into the AOTF and deflects the zero order (fluorescent light) towards the detector DE.

[00012] As a result of the angle between the first and zero orders and different wavelengths in advantageous manner a clear separation of the wavelength fractions is possible.

[00013] The invention can be used with particular advantage in a laser scanning microscope with an AOTF. Other advantageous uses of another light-diffracting element for beam separation by different diffraction orders are conceivable in a microscope beam path and are advantageously included within the scope of the invention.

[00014] Thus it can be used in advantageous manner for regulating the excitation intensity.

[00015] In Fig. 4 several such elements, here AOTF and AOM, are advantageously provided in the laser beam path for feeding in of the laser radiation. Here, several laser lines L1 - L3 like UV, VIS or IR can be fed in simultaneously or individually with an excitation power which can be adjusted independently of each other.